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Possible involvement of Ycf33 in cyclic electron transport in *Synechocystis* sp. PCC6803

M Ohtsuka, J Oyabu, H Koike, Y Kashino, K Satoh

Faculty of Science, Himeji Institute of Technology, Harima Science Garden City, Hyogo 678-1297, Japan E-mail:hkoike@sci.himeji-tech.ac.jp

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Introduction

Hypothetical chloroplast frame (*ycf*) is defined as an open reading frame of unknown function, which is found in at least two chloroplast genomes or one chloroplast and cyanobacterial genome. The *ycf*33 is found in chloroplast genomes of red lineages (red algae, diatoms and cryptomonad) and a glaucocystophyte (Stoebe et al. 1998). The frame is also found in the nuclear genome of *Arabidopsis thaliana*, and the protein has an N-terminal extension targeting to chloroplasts (*Arabitopsis* genome initiative 2001). It is thus highly possible that Ycf33 is functioning in all kinds of chloroplasts. This suggests an important role of the protein in chloroplast metabolism. Deduced amino acid sequence shows that the protein has a molecular mass of 7.5 kDa and two membane spanning regions, but it has no prominent motifs.

In the present study, we constructed a *ycf*33 deletion mutant in *Synechocystis* sp. PCC6803, and characterized the mutant. It was found that the cyclic electron transport around photosystem I is greatly affected in the mutant and the main pathway for that seems to be changed between the wild type and the mutant.

Materials and Methods

A DNA fragment of 970 bp containing *ycf*33 was amplified by PCR. The fragment was ligated to a T-A cloning vector (pGEMT-vector, Promega) and the coding region was replaced by kanamycin cassette. *Synechocystis* 6803 wild type cells were transformed by the plasmid. The transformants were picked up and a sucessive transplantation was performed on kanamycin-containig BG11 plate until seggregation was completed. The cells were grown in BG11 medium with aeration containing 5% CO_2 under various light conditions.

Absorption spectra were measured with a Shimadzu MPS-2000 spectrophotometer. Fluorescence emission spectra at 77K were recorded by a loboratory-constructed set up (Yamane et al. 1997). Redox changes of P700 were measured by monitoring the absrobance changes at 810 nm *minus* 860 nm with PAM101/103 (Walz, Gremany).

Results

Figure 1 compares absorption spectra of the wild type and the mutant. The relative absorbance at 630 nm to that at 678 nm was almost the same in the wild type, while it was higher in the *ycf*33 deletion mutant, indicative of a higher content of phycobilisomes relative to chlorophyll. The fluorescence emission spectra, when excited both chlorophyll and phycobilisomes, showed that fluorescence intensities at around 650 to 660 nm are not very high, but the intensities at 685 and 695 nm were largely increased relative to that of 725 nm in

the mutant (Fig. 2). This indicates that excitation energy absorbed by phycobilisomes are efficiently transferred to PSII, and that the PSII content relative to PSI is increased in the mutant.

It has been reported that the PSI/PSII ratio changes depending on the light intensity used to grow the cells in cyanobacteria (Murakami and Fujita, 1991). Under low intensities of light, the PSII content is decreased while it is increased under high light. When the mutant was grown under low light (20 $\mu E/m^2 \cdot s$), the absorption due to phycobilisomes (A_{630}) was decreased, and the cells grown under high light (300 $\mu E/m^2 \cdot s$) showed increased absorbance at 630nm. The extent of the decrease in the absorbance at 630 nm in the mutant was more than twice of that in the wild type (data not shown). This suggests that the high light intensity used in the present study is far higher to the mutant than to the wild type. In fact, when the mutant was grown under high light, it grew at the same rate as the wild type at first, but after two days, it started to loose its color.

These results suggest that high light intensity causes a shift in the redox level of plastoquinone (PQ) pool to a more oxidized state in the mutant. This results in an increase in the relative amount of PSII (Murakami and Fujita 1988). The redox level of PQ pool is determined by 1) electron input by PSII, 2) electron withdrawal by PSI or 3) cyclic electron

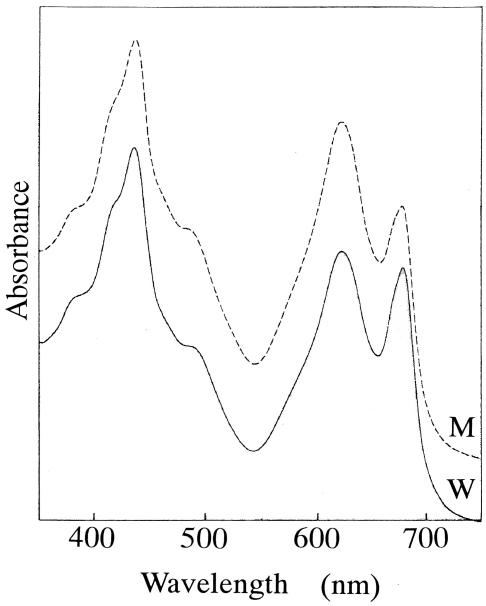


Fig. 1 Absorption spectra of wild type (W) and the mutant (M)

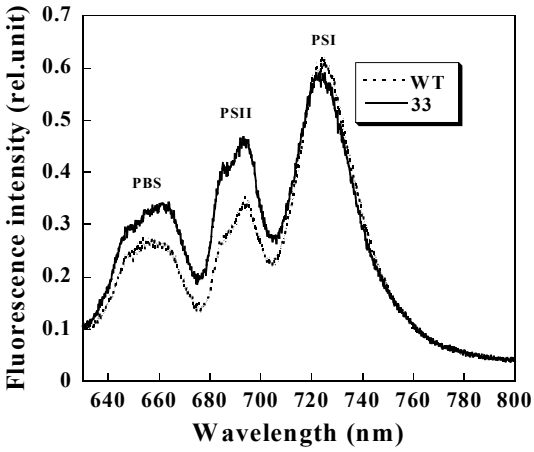


Fig. 2 Fluorescence emission spectra of the wild type (broken line) and the mutant (solid line) at 77K

transport around PSI. PSII electron transport activities determined by oxygen yield by flash was not decreased but rather increased by about 15% in the mutant. Contents of P700 were almost the same in the wild type and the mutatnt (data not shown). From these results, we can exclude the first two possibilities, and assume that a pathway of cyclic electron transport is affected in the mutant.

Cyclic electron transport activities could be monitored by measuring light-induced absorbance changes of P700 in the presence of DCMU. The re-reduction rate of P700 was not very much affected by the addition of DCMU in both the the wild type and the mutant (data not shown). Figure 3 compares redox changes of P700 in the presence of diphenyleneiodinium (DPI) and DCMU in the wild type and the mutant. DPI retarded the re-reduction rate in the wild type, while it was not much affected in the mutant. DPI is reported

to inhibit flavoprotein (O'Donnell et al. 1993). The results suggest that the wild type utilizes a pathway of cyclic electron transport in which flavoprotein participate, while the mutant uses another pathway. Judging from the reactivity of DPI, it may inhibit cyclic electron transport at FNR as well as NDH (NAD(P)H dehydrogenase) or NQR (NAD(P)H Quinone Reductase). This suggests that the cyclic electron flow is mediated by FNR in the wild type, while electrons are transferred to PQ through the site before FNR, possibly at Fd in the mutant. If this is the case, nitrite should affects the re-reduction rate very much since it accepts electrons from Fd, competing with cyclic electron transport.

Figure 4 shows effects of nitrite on the re-reduction rate of P700 in the presence of DPI and DCMU. The rate was decreased in the presence of DPI, but further addition of nitrite did not affect very much in the wild type. On the other hand, nitrite retarded the re-reduction very

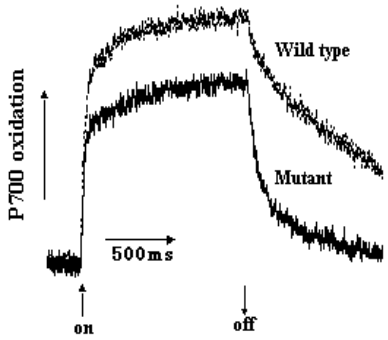
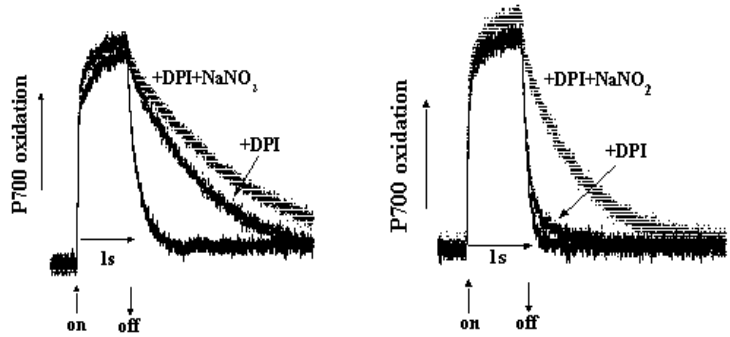


Fig. 3 Effects of 10 μM DPI on re-reduction rate of P700 in the presence of 5μM DCMU on cyclic electron transport in wild type and the mutant

Fig. 4 Effects of $NaNO_2$ on re-reduction of P700 in the presence of DCMU and DPI in the wild type (left) and the mutant (right)



much in the mutant. The results indicate that the wild type utilizes a pathway that FNR (and possibly NDH) participates, while the mutant utilizes another pathway where electrons are transferred through Fd but not FNR.

Discussion

The cyclic electron transport activity around PS I is rather high in cyanobcteria. However, its pathway is not conclusively elucidated and several pathways have been suggested (Kallas 1994). In the present study, it was indicated that the main pathway of cyclic electron transport is different between the wild type and the *ycf*33 deletion mutant. The electrons return to P700 via FNR (and possibly through NDH) in the wild type, while they return via Fd

(possibly through Ferredoxin-Quinone Reductase (FQR)). FQR has been suggested to be functioning in higher plant chloroplasts (Moss and Bendall 1984), but the existence of the protein has not been reported so far in cyanobacteria.

In both the wild type and mutant, FNR should be functioning in the cell, since the rate of photosynthesis was not much different between them. However, when *ycf*33 is deleted from the cell, FNR does not participate in cyclic electron transport. Then, a question arises on functional relationship between FNR and Ycf33. FNR of *Synechocystis* 6803 has an N-terminal extension homologous to CpcD (Kaneko et al. 1996), which is a linker polypeptide of phycobilisomes. It has been reported that cyclic electron transport in a mutant, in which the N-terminal extension of FNR had been deleted, was greatly suppressed (van Thor et al. 2000). This suggests that FNR is somehow connected to thylakoid membranes by the extension in the wild type and transfers electrons to PQ pool in the cyclic pathway. On the other hand, when FNR is detached from the thylakoid membranes by deletion of the extension in the mutant, the enzyme could not transfer electrons to PQ. However, the extension does not have any hydrophobic segments, so that the enzyme should interact with other component which is buried in the thylakoid membranes. A possible candidate for that is Ycf33, since it has two membrane spanning regions. When Ycf33 is deleted, FNR could not be anchored to the membranes and the cyclic electron transport pathway via FNR could not be used in the mutant. Another pathway from Fd to the PQ pool, possibly via FQR, is supposed to be the main pathway in the mutant.

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